UNIVERSITY OF PENNSYLVANIA

SEROLOGIC AND CULTURAL STUDIES OF MENINGOCOCCI

WITH SPECIAL REFERENCE TO TYPE V

BESS E. SEGAL

A THESIS

IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Since the first appearance of meningococcic meningitis on the Pacific coast in 1928, outbreaks have occurred throughout the United States with increasing frequency. The rate of incidence of epidemic meningitis was higher in 1928, 1929 and 1930 than in any other year since 1918. In these recent outbreaks, it was reported that available therapeutic serums did not give results as satisfactory as had been expected from previous experience. There was, in these recent epidemics, a high mortality among serum treated persons. For this ineffectiveness of therapeutic serums several explanations have been advanced. It has been thought that the strains used in the production of the serums might have lost their antigenic specificity, or that there were existent in these outbreaks strains different from the usual types of meningococci encountered heretofore.

Numerous cases of cerebrospinal meningitis are known to have occurred in the Orient, resulting in many deaths. The oriental disease is virulent, the mortality being high in spite of treatment with serum. The development of modern transportation and industry have made conditions such that the causative agent of the oriental outbreaks may have been brought to the Pacific coast.

The following study of the types of meningococci was made in an effort to discover whether there existed any new types among cultures isolated from cases in recent epidemics.

Since May, 1929, one hundred cultures of meningococci were received for study from various sources. Thirty-eight strains were received from the Department of Health, Chicago, through the courtesy of the National Institute of Health, Washington, D. C.; twenty-seven from Dr. C. Y. White, of the Municipal Hospital, Philadelphia; twenty-four from Dr. P. S. Rhoads, of the John McCormick Institute for Infectious

* From the Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa., and the Laboratory of Hygiene of the University of Pennsylvania, Philadelphia.

Diseases, Chicago; five from Dr. J. F. Norton, Department of Health, Detroit; one from Dr. H. M. Heise, of Uniontown, Pa.; three from Dr. Benjamin White, of the Department of Health, Boston; one from Dr. B. I. Krehbiel, of the City Isolation Hospital, St. Louis; two from the Lankenau Hospital, Philadelphia, and two from Dr. Karl Meyer, of the University of California Medical School, San Francisco. All of these hundred strains were isolated from the spinal fluids in cases of cerebrospinal meningitis. All were gram-negative diplococci, and according to growth and colony formation were considered indistinguishable from the four Gordon type strains of meningococci used by the National Institute of Health, Washington.

For the production of monovalent serums, the antigens used were eighteen hour cultures on hog liver agar, suspended in buffered saline solution; 0.3 per cent formalin was added and the suspension kept at room temperature for three days. Rabbits weighing about 2,000 Gm. were tested for the presence of normal agglutinins against the strain to be used in immunization and the four Gordon type strains. Injections were given intravenously twice a week; the first dose was 0.2 cc.; the total amount injected did not exceed 1.5 cc. The rabbits were bled five days after the last injection. In immunizing rabbits with the four type strains, high titers were sacrificed for specificity. I found it difficult to obtain a highly specific serum with any strain, even on the injection of only a few doses.

In making suspensions for agglutination tests, eighteen hour cultures on hog liver agar were used. Saline solution was used as the diluent. The organisms were heat-killed at 56 C. for one hour and standardized to 2,000,000,000 per cubic centimeter. The tubes were incubated in a water bath for four hours at 56 C. and were read immediately thereafter. Antigen and serum controls were included in each test.

Absorption tests were performed by adding four parts of a suspension of the antigen 50,000,000,000 per cubic centimeter to one part of the serum, both having been previously chilled. The mixture was shaken, and the tubes were placed in the water bath at 37.5 C. for fifteen minutes, and shaken frequently. To separate the organisms from the absorbed serum, the tubes were centrifugated at high speed.

The hundred strains of meningococci were all tested against monovalent serums prepared as described. Seventy-eight strains, by virtue of their agglutination and absorption reactions, were grouped, according to

the classification of Gordon and Murray, as follows: type I, thirty-six; type II, ten; type III, twenty, and type IV, twelve. Twenty-two strains were not agglutinated by any of the monovalent serums used, even in low dilutions. Five of these, nos. 125, 126, 127, 139 and 204, were, however, agglutinated by polyvalent serums in sufficiently high dilutions to be considered specific for the species. Seventeen strains showed little or no agglutination with polyvalent serums (see table).

Strains 125, 126, 127, 139 and 204 were grown in hormone broth with 5 per cent ascitic fluid in an attempt to increase their agglutin-

Serologic and Fermentative Reactions of Seventeen Strains of Meningococcus Type V and of Gordon Type Strains

Antigens	Titer of Antigen Control	Titer of Normal Serum Control	Titer of Monovalent Rabbit Serum Prepared Against					Titer of Polyvalent Horse Serum	Carbohydrate Fermentation		
			Type I (123)	Type II (55)	Type III (57)	Type IV (60)	Type V (223)		Maltose	Dextrose	Saccharose
128	0	0	0	0	0	0	640	20	+	—	+
129	0	0	0	0	0	0	1280	0	+	—	—
155	0	0	0	0	0	0	1280	40	+	—	—
156	0	0	0	0	0	0	1280	40	+	—	—
157	0	0	0	0	0	0	640	0	—	—	—
158	0	0	0	0	0	0	1280	20	+	+	+
159	0	0	0	0	0	0	640	20	+	+	—
160	0	0	0	0	0	0	1280	20	+	+	—
208	0	0	0	0	0	0	640	40	+	—	—
209	0	0	0	0	0	0	320	0	—	—	—
216	0	0	0	0	0	0	1280	40	—	—	—
217	0	0	0	0	0	0	1280	40	—	—	—
218	0	0	0	0	0	0	160	40	—	—	—
219	0	0	0	0	0	0	1280	40	+	+	—
221	0	0	0	0	0	0	1280	40	—	—	—
223	0	0	0	0	0	0	1280	0	—	—	+
225	0	0	0	0	0	0	1280	20	—	—	—
Gordon type strains I (123)	0	0	320	0	20	20	0	320	+	+	—
Gordon type strains II (55)	0	0	0	320	10	20	0	320	+	+	—
Gordon type strains III (57)	0	0	0	20	160	20	0	320	+	+	—
Gordon type strains IV (60)	0	0	0	40	0	640	0	320	+	+	—

ability. After seven daily transplants, two strains were agglutinated by the antiserum of type IV. Rabbits were immunized with all five strains and the agglutination reactions of their antiserums were studied. Many doses were required to obtain an antiserum of sufficiently high agglutinin titer for the homologous strain. The antiserums from four strains (nos. 125, 126, 127 and 204) agglutinated not only the homologous strain, but also the Gordon type IV strain. The antiserum of strain 139 agglutinated the Gordon type III strain. These results were confirmed by cross-absorptions.

Of the seventeen strains agglutinated in low dilutions only, or not at all, by polyvalent serums, strain 223, selected at random, was used in

immunizing rabbits. All of the seventeen strains were found agglutinable by the antiserum of strain 223 in high dilutions, while no agglutination of the four Gordon type strains was obtained with this antiserum even in the dilution 1 : 10 (see table).

Complete absorption of agglutinins from the antiserum of strain 223 was effected by each of the seventeen strains. There was no absorption of agglutinins from the antiserum of strain 223 by the four Gordon type strains, neither would strain 223 absorb agglutinins from the antiserums of the four Gordon type strains.

Thus, by absorption and agglutination tests, these seventeen strains were found to form a homogeneous antigenic group serologically unrelated to the four Gordon type strains.

According to morphology and cultural and staining reactions, I consider these seventeen strains indistinguishable from the Gordon type strains. They grow equally well on all the mediums employed, namely, blood agar, ascitic agar, hormone agar, dextrose agar, semisolid agar, Loeffler's blood serum and hog liver agar. On semisolid medium, these seventeen strains all produce a yellowish pigment, the color varying in intensity with the different strains. Among the Gordon type strains, there are many that produce pigment. Some type I and III strains in our collection produce a pale yellow or pinkish colony, while type II and IV strains tend to produce a yellow pigment that deepens with age. On Loeffler's blood serum, the seventeen strains produced pigment varying from deep yellow to greenish-yellow. The type strains were white, tan or slightly yellow. Trautman [1] observed that certain strains of meningococci are capable of producing a greenish-yellow pigment on Loeffler's blood serum. Atkins [2] described the appearance of a yellow pigment in older colonies of meningococci grown on trypagar.

The carbohydrate fermentation reactions of these seventeen strains were studied, "Difco" maltose, dextrose and saccharose being used. The sugar solutions were sterilized by filtration through Berkefeld filters. The sugar solutions were added aseptically to previously sterilized sugar-free agar to make it 1 per cent carbohydrate. Andrade's solution was used as indicator. Enlow's fermentation medium was also used. The sugars for this medium were added to sugar-free agar to make it 0.5 per cent carbohydrate, bromthymol blue being used as indicator. It was observed that the seventeen strains do not form a homogeneous group as regards

1. Trautman, cited by Elser and Huntoon: J. M. Research **208**:373, 1909.
2. Brit. J. Exper. Path. **6**:325, 1923.

carbohydrate fermentations. Some strains were found to ferment maltose only; others, maltose and saccharose. One strain fermented saccharose only; three strains fermented maltose and dextrose. Seven of these strains did not ferment any of the sugars (see table). Tests were repeated on several lots of mediums. With each lot, Bacillus coli-communis, B. coli-communior and the four meningococcus type strains were used as controls. Somewhat variable fermentation reactions were found with every test. Branham [3] studied the fermentation reactions of fourteen strains isolated from cases of meningitis in the Chicago epidemic of 1928, and reported constantly negative results as regards sugar fermentations for these fourteen strains. Branham considered the pigmentation and the fermentation reaction of the fourteen strains as sufficiently important and distinct from those of the four Gordon type strains to warrant placing the fourteen strains apart as a new species. Branham proposed for these organisms the name Neisseria flavescens, U. S. P. Cameron [4] found irregular fermentation reactions comparable to my findings among twenty-seven atypical strains isolated from cases of meningitis in the same epidemic. Variations have been found among the type strains of meningococci. Sophian [5] described strains of meningococci that ferment dextrose or maltose and some that ferment no sugars. Arkwright,[6] Scott [5] and Griffith [5] found these capricious fermentation reactions to occur especially with freshly isolated cultures. Norton and Broom,[7] working with ninety-five strains of meningococci isolated from cases in the Detroit epidemic, found that carbohydrate fermentation tests with maltose gave the most consistent results, that several strains did not ferment dextrose, and that saccharose fermentation was not uncommon.

SUMMARY

Of the hundred strains of meningococci studied, thirty-six strains by agglutination and absorption tests were found to be type I, ten strains type II, twenty type III and twelve type IV.

An indirect method of classification was resorted to in five cases. Rabbits were immunized with each of the strains, and the agglutination

3. U. S. Pub. Health Rep. **45**:16, 1930.

4. Canad. Pub. Health J. **21**:290, 1930.

5. Cited by Murray: The Meningococcus, Medical Research Council, Report 124, 1929.

6. J. Hyg. **9**:104, 1909.

7. J. Prev. Med. **4**:355, 1930.

and absorption reactions of the homologous antiserums were studied. Four were found to be of type IV and one of type III.

Seventeen strains were found to differ serologically from any of the known types of meningococci. They have been shown to form a separate group distinguishable from the Gordon type strains by their agglutination and absorption reactions.

The cultural and biochemical reactions of these seventeen strains I do not consider as sufficiently distinct to warrant their being placed apart as a new species. A fifth type is proposed for this group of seventeen strains, since the four existing Gordon types are differentiated purely on the basis of serologic differences.

The seventeen strains forming a fifth type of meningococcus were all isolated from the spinal fluids in cases of clinical cerebrospinal meningitis occurring in the Chicago epidemic of 1928, in which all the four known Gordon types were encountered.